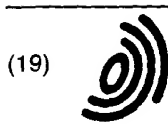


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(71) Applicants:  
 • **SMITHKLINE BEECHAM CORPORATION**  
 Philadelphia Pennsylvania 19103 (US)  
 • **SMITHKLINE BEECHAM PLC**  
 Brentford, Middlesex TW8 9EP (GB)

(72) Inventors:  
 • **Powell, David J.**  
 King of Prussia, Pennsylvania 19406 (US)

- **Southan, Christopher,**  
 SmithKline Beecham Pharm.  
 Harlow, Essex, CM19 5AW (GB)
- **Chapman, Conrad G.,**  
 SmithKline Beecham Pharm.  
 Harlow, Essex, CM19 5AW (GB)
- **Evans, Joanne R.,** SmithKline Beecham Pharm.  
 Harlow, Essex, CM19 5AW (GB)

(74) Representative:  
**Connell, Anthony Christopher et al**  
 SmithKline Beecham plc  
 Corporate Intellectual Property,  
 Two New Horizons Court  
 Brentford, Middlesex TW8 9EP (GB)

(54) **Aspartic protease ASP1**

(57) ASP1 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are

methods for utilizing ASP1 polypeptides and polynucleotides in the design of protocols for the treatment of Alzheimers's Disease, cancer, and melanoma, among others, and diagnostic assays for such conditions.

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**Description****FIELD OF INVENTION**

5 This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to the Aspartic Proteinase family, hereinafter referred to as ASP1. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

**BACKGROUND OF THE INVENTION**

There are currently five known human aspartic proteases, namely pepsin, gastricsin, cathepsin D, cathepsin E and renin, and these have widely varying functions. Pepsin and gastricsin are involved in nutritive processes in the stomach, cathepsin D is involved in protein turnover in many cell types, and renin has the highly specific function of angiotensin production from its precursor form, angiotensinogen. The precise role of cathepsin E remains to be confirmed, although its location in some epithelial cells types has indicated a role in antigen processing. It may also be involved in certain inflammatory conditions, e.g. *Helicobacter pylori* infection in the stomach. This indicates that the Aspartic Proteinase family has an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further members of the Aspartic Proteinase family which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, Alzheimers's Disease, cancer, and melanoma.

**SUMMARY OF THE INVENTION**

25 In one aspect, the invention relates to ASP1 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such ASP polypeptides and polynucleotides. Such uses include the treatment of Alzheimers's Disease, cancer, and melanoma, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with ASP1 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate ASP1 activity or levels.

**DESCRIPTION OF THE INVENTION****Definitions**

35 The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"ASP1" refers, among others, generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO: 2 or an allelic variant thereof.

40 "ASP1 activity or ASP1 polypeptide activity" or "biological activity of the ASP1 or ASP1 polypeptide" refers to the metabolic or physiologic function of said ASP1 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said ASP1.

"ASP 1 gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

45 "Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

50 "Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes 55 DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or met-

abolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al.*, *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al.*, *J Molec Biol* (1990) 215:403).

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations

per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5 or 3 terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

### Polypeptides of the Invention

In one aspect, the present invention relates to ASP1 polypeptides (or ASP1 proteins). The ASP1 polypeptides include the polypeptide of SEQ ID NO:2; as well as polypeptides comprising the amino acid sequence of SEQ ID NO: 2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within ASP1 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and still more preferably at least 95% identity to SEQ ID NO:2. Furthermore, those with at least 97-99% are highly preferred. Preferably ASP1 polypeptide exhibit at least one biological activity of ASP1.

The ASP1 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the ASP1 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned ASP1 polypeptides. As with ASP polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of ASP1 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of ASP1 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate ASP1 activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the ASP1, including antigenic activity. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The ASP1 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

### Polynucleotides of the Invention

Another aspect of the invention relates to ASP1 polynucleotides. ASP1 polynucleotides include isolated polynucleotides which encode the ASP1 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, ASP1 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 encoding a ASP1 polypeptide of SEQ ID NO:2, and polynucleotide having the particular sequence of SEQ ID NO: 1. ASP1 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the ASP1 polypeptide of SEQ ID NO:2, and a polynucleotide comprising a nucleotide sequence that is at least 80% identical to of SEQ ID NO: 1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under ASP1 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO: 1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such ASP1 polynucleotides.

ASP1 of the invention is structurally related to other proteins of the Aspartic Proteinase family, as shown by the results of sequencing the cDNA of Table 1 (SEQ ID NO: 1 ) encoding human ASP1. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 91 to 1644) encoding a polypeptide of 518 amino acids of SEQ ID NO:2. The amino acid sequence of Table 2 (SEQ ID NO:2) has about 50% identity (using FASTA (GCG)) in 445 amino acid residues with ASP2 Novel Aspartic Proteinase (UK Pat. Refs 9618966.7, 9618966.6 and 9618963.4). Furthermore, human progastricsin is 33.5% identical to ASP1 over 239 amino acid residues (Taggart, R.T. et al., J. Biol. Chem. 264:375-379, 1989). The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 60.4% identity (using FASTA (GCG)) in 1347 nucleotide residues with ASP2 Novel Aspartic Proteinase (UK Pat. Refs. 9618966.7, 9618966.6 and 9618963.4). Furthermore, human progastricsin is 45% identical to ASP1 over 1385 nucleotide base residues (Taggart et al., J. Biol. Chem. 264:375-379, 1989). Thus, ASP1 polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

Table 1<sup>a</sup>

GGCCGCTGAATGGCCGAGTCGCTGAGCCGCGGCTGCCGGACGGGACGGGACCGGCTAG  
5 GCTGGGCGCGCCCCCGGGCCCCGCGTGGGCATGGGCGCACTGGCCCGGGCGCTGCT  
GCTGCCTCTGCTGGCCCAGTGGCTCCTGCGCGCCGCCCCGGAGCTGGCCCCCGCGCCC  
10 TTCACGCTGCCCCCTCCGGGTGGCCGCGGCCACGAACCGCGTAGTTGCGCCCACCCCGG  
GACCCGGGACCCCTGCCGAGCGCCACGCCGACGGCTTGGCGCTCGCCCTGGAGCCTGC  
CCTGGCGTCCCCCGCGGGCGCCGCAACTTCTTGGCCATGGTAGACAACCTGCAGGGG  
15 GACTCTGGCCGCGGCTACTACCTGGAGATGCTGATCGGGACCCCCCGCAGAAGCTAC  
AGATTCTCGTTGACACTGGAAGCAGTAACTTTGCCGTGGCAGGAACCCCGCACTCCTA  
CATAGACACGTACTTTGACACAGAGAGGTCTAGCACATACCGCTCCAAGGGCTTTGAC  
20 GTCACAGTGAAGTACACACAAGGAAGCTGGACGGGCTTCGTTGGGGAAGACCTCGTCA  
CCATCCCCAAAGGCTTCAATACTTCTTTTCTTGTCAACATTGCCACTATTTTGAATC  
AGAGAATTTCTTTTGCCTGGGATTAAATGGAATGGAATACTTGGCCTAGCTTATGCC  
25 ACACTTGCCAAGCCATCAAGTTCTCTGGAGACCTTCTTCGACTCCCTGGTGACACAAG  
CAAACATCCCCAACGTTTTCTCCATGCAGATGTGTGGAGCCGGCTTGCCCGTTGCTGG  
ATCTGGGACCAACGGAGGTAGTCTTGTCTTGGGTGGAATTGAACCAAGTTTGTATAAA  
30 GGAGACATCTGGTATACCCCTATTAAGGAAGAGTGGTACTACCAGATAGAAATTCTGA  
AATTGGAAATTGGAGGCCAAAGCCTTAATCTGGACTGCAGAGAGTATAACGCAGACAA  
35 GGCCATCGTGGACAGTGGCACCACGCTGCTGCGCCTGCCCCAGAAGGTGTTTGATGCG  
GTGGTGGAAAGCTGTGGCCCCGCGCATCTCTGATTCCAGAATTCTCTGATGGTTTCTGGA

CTGGGTCCCAGCTGGCGTGCTGGACGAATTCGGAACACCTTGGTCTTACTTCCCTAA  
 AATCTCCATCTACCTGAGAGACGAGAACTCCAGCAGGTCATTCCGTATCACAATCCTG  
 CCTCAGCTTTACATTACAGCCCATGATGGGGGCCCGCCTGAATTATGAATGTTACCGAT  
 TCGGCATTTCCCCATCCACAAATGCGCTGGTGATCGGTGCCACGGTGATGGAGGGCTT  
 CTACGTCATCTTCGACAGAGCCCAGAAGAGGGTGGGCTTCGCAGCGAGCCCCTGTGCA  
 GAAATTGCAGGTGCTGCAGTGTCTGAAATTTCCGGGCCTTTCTCAACAGAGGATGTAG  
 CCAGCAACTGTGTCCCCGCTCAGTCTTTGAGCGAGCCCATTTTGTGGATTGTGTCCTA  
 TGCGCTCATGAGCGTCTGTGGAGCCATCCTCCTTGTCTTAATCGTCCTGCTGCTGCTG  
 CCGTTCCGGTGTGAGCGTCGCCCCGTGACCCTGAGGTCGTCAATGATGAGTCCTCTC  
 TGGTCAGACATCGCTGGAAATGAATAGCCAGGCCTGACCTCAAGCAACCATGAACTCA  
 GCTATTAAGAAAATCACATTTCCAGGGCAGCAGCCGGGATCGATGGTGGCGCTTTCTC  
 CTGTGCCCCACCGTCTTCAATCTCTGTTCTGCTCCCAGATGCCTTCTAGATTCACTGT  
 CTTTTGATTCTTGATTTTCAAGCTTTCAAATCCTCCCTACTTCCAAGAAAAAAAAAA  
 AAAAAA

<sup>a</sup> A nucleotide sequence of a human ASP1 (SEQ ID NO: 1).

**Table 2<sup>b</sup>**

MGALARALLPLLAQWLLRAAPELAPAPFTLPLRVAAATNRVVAPTGPGPPTPAERHAD  
 GLALALEPALASPAGAAFLAMVDNLQDSSGRGYYLEMLIGTPPQKLQILVDTGSSNF  
 AVAGTPHSYIDTYFDTERSSTYRSKGFDTVKYTQGSWTGFVGEDLVTIPKGFNTSFL  
 VNIATIFESENFFLPGIKWNGILGLAYATLAKPSSSLETFFDSLVTQANI PNVFSMQM  
 CGAGLPVAGSGTNGGSLVLGGIEPSLYKGDWYTPIKEEWWYQIEILKLEIGGQSLNL  
 DCREYNADKAIVDSGTTLLRLPQKVFDVAVEAVARASLIPEFSDGFWTGSQACWNTS  
 ETPWSYFPKISIIYLRDENSSRSFRITILPQLYIQPMMGAGLNYECYRFGISPSTNALV  
 IGATVMEGFYVIFDRAQKRVGFAASPCAIEIAGAASEISGPFSTEDVASNCVPAQSLS  
 EPILWIVSYALMSVCGAILLVLLVLLLLPFRQCRRPRDPEVVNDESSLVRHRWK

<sup>b</sup> An amino acid sequence of a human ASP1 (SEQ ID NO: 2).

One polynucleotide of the present invention encoding ASP1 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human Melanocyte, melanoma, endothelial cells, adult brain, and fibroblasts using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature*, (1992) 355:632-634; Adams, M.D., *et al., Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding ASP1 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 91 to 1644 of SEQ ID NO:1), or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of ASP1 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain noncoding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding ASP1 variants comprise the amino acid sequence ASP1 polypeptide of Table 2 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination.

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more preferably 97-99% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO: 1 or a fragment thereof, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding ASP1 polypeptide and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than human) that have a high sequence similarity to the ASP1 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

In one embodiment, to obtain a polynucleotide encoding ASP1 polypeptide, including homologs and orthologs from species other than human, comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof; and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Thus in another aspect, ASP1 polynucleotides of the present invention further include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having SEQ ID NO: 1 or a fragment thereof. Also included with ASP1 polypeptides are polypeptide comprising amino acid sequence encoded by nucleotide sequence obtained by the above hybridization condition. Such hybridization techniques are well known to those of skill in the art. Stringent hybridization conditions are as defined above or, alternatively, conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

### Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis *et al.*, *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as



*Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the ASP1 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If the ASP1 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

ASP1 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

#### Diagnostic Assays

This invention also relates to the use of ASP1 polynucleotides for use as diagnostic reagents. Detection of a mutated form of an ASP1 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression, or altered expression of ASP1. Individuals carrying mutations in the ASP1 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled ASP1 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotide probes comprising ASP1 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee *et al.*, *Science*, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to Alzheimer's Disease, cancer, and melanoma through detection of mutation in the ASP 1 gene by the methods described.

In addition, Alzheimer's Disease, cancer, and melanoma can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of ASP1 polypeptide or ASP1 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an ASP1 polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagnostic kit for a disease or susceptibility to a disease,

particularly Alzheimers's Disease, cancer, and melanoma, which comprises:

- (a) a ASP1 polynucleotide, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof;
- (b) a nucleotide sequence complementary to that of (a);
- (c) a ASP1 polypeptide, preferably the polypeptide of SEQ ID NO: 2, or a fragment thereof; or
- (d) an antibody to a ASP1 polypeptide, preferably to the polypeptide of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

#### Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

The chromosomal localization of ASP1 has been identified as 21q22 (Down's Critical Region) by radiation hybridization mapping.

#### Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the ASP1 polypeptides. The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the ASP1 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against ASP1 polypeptides may also be employed to treat Alzheimers's Disease, cancer, and melanoma, among others.

#### Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with ASP1 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from Alzheimers's Disease, cancer, and melanoma, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises delivering ASP1 polypeptides via a vector directing expression of ASP1 polynucleotides *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to an ASP1 polypeptide wherein the composition comprises an ASP1 polypeptide or ASP1 gene. The vaccine formulation may further comprise a suitable carrier. Since ASP1 polypeptides may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral

administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

## Screening Assays

The ASP polypeptide of the present invention may be employed in a screening process for compounds which activate (agonists) or inhibit activation of (antagonists, or otherwise called inhibitors) the ASP1 polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess identify agonist or antagonists from, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These agonists or antagonists may be natural or modified substrates, ligands, receptors, enzymes, etc., as the case may be, of the polypeptide of the present invention; or may be structural or functional mimetics of the polypeptide of the present invention. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

ASP1 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirable to find compounds and drugs which stimulate ASP1 polypeptides on the one hand and which can inhibit the function of ASP1 polypeptides on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as Alzheimer's Disease, cancer, and melanoma. Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as Alzheimer's Disease, cancer, and melanoma.

In general, such screening procedures may involve using appropriate cells which express the ASP polypeptide or respond to ASP1 polypeptide of the present invention. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells which express the ASP1 polypeptide (or cell membrane containing the expressed polypeptide) or respond to ASP1 polypeptide are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. The ability of the cells which were contacted with the candidate compounds is compared with the same cells which were not contacted for ASP1 activity.

All aspartic proteinases are inhibited by pepstatin.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the ASP1 polypeptide is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the ASP1 polypeptide, using detection systems appropriate to the cells bearing the ASP1 polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed.

Further, the assays may simply comprise the steps of mixing a candidate compound with a solution containing a ASP1 polypeptide to form a mixture, measuring ASP1 activity in the mixture, and comparing the ASP1 activity of the mixture to a standard.

The ASP1 cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of ASP1 mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell associated levels of ASP1 protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents which may inhibit or enhance the production of ASP1 (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The ASP1 protein may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. These include, but are not limited to, ligand binding and crosslinking assays in which the ASP1 is labeled with a radioactive isotope (eg 125I), chemically modified (eg biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (cells, cell membranes, cell supernatants, tissue extracts, bodily fluids). Other methods include biophysical techniques such as surface plasmon resonance and spectroscopy. In addition to being used for purification and cloning of the receptor, these binding assays can be used to identify agonists and antagonists of ASP1 which compete with the binding of ASP1 to its receptors, if any. Standard methods for conducting screening assays are well understood in the art.

Examples of potential ASP1 polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, receptors, enzymes, etc., as the case may be, of the ASP1 polypeptide, e.g., a fragment of the ligands, substrates, receptors, enzymes, etc.; or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for ASP1 polypeptides; or compounds which decrease or enhance the production

of ASP1 polypeptides, which comprises:

- (a) an ASP1 polypeptide, preferably that of SEQ ID NO:2;
- (b) a recombinant cell expressing an ASP1 polypeptide, preferably that of SEQ ID NO:2;
- (c) a cell membrane expressing an ASP1 polypeptide; preferably that of SEQ ID NO: 2; or
- (d) antibody to an ASP1 polypeptide, preferably that of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

## Prophylactic and Therapeutic Methods

This invention provides methods of treating abnormal conditions such as Alzheimer's Disease, cancer, and melanoma, related to both an excess of and insufficient amounts of ASP1 polypeptide activity.

If the activity of ASP1 polypeptide is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit the function of the ASP1 polypeptide, such as, for example, by blocking the binding of ligands, substrates, receptors, enzymes, etc., or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of ASP1 polypeptides still capable of binding the ligand, substrate, enzymes, receptors, etc. in competition with endogenous ASP1 polypeptides may be administered. Typical embodiments of such competitors comprise fragments of the ASP1 polypeptide.

In still another approach, expression of the gene encoding endogenous ASP1 polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL II 988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of ASP and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates ASP1 polypeptide, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of ASP1 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of ASP1 polypeptides in combination with a suitable pharmaceutical carrier.

## Formulation and Administration

Peptides, such as the soluble form of ASP1 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

### Examples

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

#### Example 1

Nucleotides 1-285 of the full length ASP1 sequence were isolated from human melanoma Marathon-Ready™ cDNA, available from Clontech Laboratories Inc. (Palo Alto, CA USA). Marathon-Ready™ cDNAs are essentially cDNA libraries which have oligonucleotide adaptors ligated onto their ends. This allows the researcher to perform RACE (rapid amplification of cDNA ends) PCR using two primers, one complementary to a region of known sequence in the gene of interest and the other complementary to the ligated adaptor; resulting in an extension to the known gene sequence. RACE PCR can be performed at either the 5' or the 3' end of the gene. In this case, RACE PCR was performed on the 5' end.

Due to the very high GC content of the 5' end of the ASP1 gene it was found necessary to use the Advantage-GC™ cDNA PCR kit from Clontech for performing the PCR. It was also found necessary to use nested PCR - this is a second PCR which uses primers internal to the first pair and uses the first PCR product as a template.

The final PCR product was subcloned into the pTarget™ vector, from Clontech, for DNA sequencing.

Nucleotides 286-1862 have been collated from the sequencing of available Expressed Sequence Tags (ESTs). A full length clone was obtained by ligating the RACE PCR fragment to an overlapping EST sequence.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

SEQUENCE LISTING

5

(1) GENERAL INFORMATION

10

(i) APPLICANT: SmithKline Beecham Corporation

(ii) TITLE OF THE INVENTION: ASP1

15

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

20

(A) ADDRESSEE: SmithKline Beecham, Corporate Intellectual  
Property

(B) STREET: Two New Horizons Court

(C) CITY: Brentford

25

(D) STATE: Middlesex

(E) COUNTRY: United Kingdom

(F) POST CODE: TW8 9EP

30

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette

35

40

45

50

55

(B) COMPUTER: IBM Compatible  
(C) OPERATING SYSTEM: DOS  
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER: TO BE ASSIGNED  
(B) FILING DATE: 06-OCT-1997  
(C) CLASSIFICATION: UNKNOWN

(vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: CONNELL, Anthony Christopher  
(B) GENERAL AUTHORISATION NUMBER 5630  
(C) REFERENCE/DOCKET NUMBER: GH-70262

(ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: +44 127 964 4395  
(B) TELEFAX: +44 181 975 6294  
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1862 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGCCGCTGAA	TGGCCGAGTC	GCTGAGCCGC	GGCTGCCGGA	CGGGACGGGA	CCGGCTAGGC	60
TGGGCGCGCC	CCCCGGGCCC	CGCCGTGGGC	ATGGGCGCAC	TGGCCCGGGC	GCTGCTGCTG	120
CCTCTGCTGG	CCCAGTGGCT	CCTGCGCGCC	GCCCCGGAGC	TGGCCCCCGC	GCCCTTCACG	180
CTGCCCCCTC	GGGTGGCCGC	GGCCACGAAC	CGCGTAGTTG	CGCCCACCCC	GGGACCCGGG	240
ACCCCTGCCG	AGCGCCACGC	CGACGGCTTG	GCGCTCGCCC	TGGAGCCTGC	CCTGGCGTCC	300
CCCGCGGGCG	CCGCCAACTT	CTTGCCCATG	GTAGACAAAC	TGCAGGGGGA	CTCTGGCCGC	360
GGCTACTACC	TGGAGATGCT	GATCGGGACC	CCCCCGCAGA	AGCTACAGAT	TCTCGTTGAC	420
ACTGGAAGCA	GTAACTTTGC	CGTGGCAGGA	ACCCCGCACT	CCTACATAGA	CACGTACTTT	480

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5 GACACAGAGA GGTCTAGCAC ATACCGCTCC AAGGGCTTTG ACGTCACAGT GAAGTACACA 540  
 CAAGGAAGCT GGACGGGCTT CGTTGGGGAA GACCTCGTCA CCATCCCCAA AGGCTTCAAT 600  
 ACTTCTTTTC TTGTCAACAT TGCCACTATT TTTGAATCAG AGAATTTCTT TTTGCCTGGG 660  
 ATTAAATGGA ATGGAATACT TGGCCTAGCT TATGCCACAC TTGCCAAGCC ATCAAGTTCT 720  
 CTGGAGACCT TCTTCGACTC CCTGGTGACA CAAGCAAACA TCCCCAACGT TTTCTCCATG 780  
 CAGATGTGTG GAGCCGGCTT GCCCGTTGCT GGATCTGGGA CCAACGGAGG TAGTCTTGTC 840  
 10 TTGGGTGGAA TTGAACCAAG TTTGTATAAA GGAGACATCT GGTATACCCC TATTAAGGAA 900  
 GAGTGGTACT ACCAGATAGA AATTCTGAAA TTGGAAATTG GAGGCCAAAG CCTTAATCTG 960  
 GACTGCAGAG AGTATAACGC AGACAAGGCC ATCGTGGAACA GTGGCACCAC GCTGCTGCGC 1020  
 CTGCCCCAGA AGGTGTTTGA TGCGGTGGTG GAAGCTGTGG CCCGCGCATC TCTGATTCCA 1080  
 15 GAATTCTCTG ATGGTTTCTG GACTGGGTCC CAGCTGGCGT GCTGGACGAA TTCGGAAACA 1140  
 CTTTGGTCTT ACTTCCCTAA AATCTCCATC TACCTGAGAG ACGAGAACTC CAGCAGGTCA 1200  
 TTCCGTATCA CAATCCTGCC TCAGCTTTAC ATTCAGCCCA TGATGGGGGC CGGCCTGAAT 1260  
 TATGAATGTT ACCGATTCGG CATTTCCCCA TCCACAAATG CGCTGGTGAT CGGTGCCACG 1320  
 20 GTGATGGAGG GCTTCTACGT CATCTTCGAC AGAGCCCAGA AGAGGGTGGG CTTGCGAGCG 1380  
 AGCCCCGTGTG CAGAAATTGC AGGTGCTGCA GTGTCTGAAA TTTCCGGGCC TTTCTCAACA 1440  
 GAGGATGTAG CCAGCAACTG TGTCCCCGCT CAGTCTTTGA GCGAGCCCAT TTTGTGGATT 1500  
 GTGTCCTATG CGCTCATGAG CGTCTGTGGA GCCATCCTCC TTGTCTTAAT CGTCTGCTG 1560  
 25 CTGCTGCCGT TCCGGTGTCA GCGTCGCCCC CGTGACCCTG AGGTCGTCAA TGATGAGTCC 1620  
 TCTCTGGTCA GACATCGCTG GAAATGAATA GCCAGGCCTG ACCTCAAGCA ACCATGAACT 1680  
 CAGCTATTAA GAAAATCACA TTTCCAGGGC AGCAGCCGGG ATCGATGGTG GCGCTTTCTC 1740  
 CTGTGCCCCA CCGTCTTCAA TCTCTGTTCT GCTCCCAGAT GCCTTCTAGA TTCCTGTCT 1800  
 30 TTTGATTCTT GATTTTCAAG CTTTCAAATC CTCCCTACTT CCAAGAAAAA AAAAAAAAAA 1860  
 AA 1862

## (2) INFORMATION FOR SEQ ID NO:2:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 518 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

### (ii) MOLECULE TYPE: protein

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Gly Ala Leu Ala Arg Ala Leu Leu Leu Pro Leu Leu Ala Gln Trp  
 1 5 10 15  
 50 Leu Leu Arg Ala Ala Pro Glu Leu Ala Pro Ala Pro Phe Thr Leu Pro  
 20 25 30  
 Leu Arg Val Ala Ala Ala Thr Asn Arg Val Val Ala Pro Thr Pro Gly  
 35 40 45  
 55 Pro Gly Thr Pro Ala Glu Arg His Ala Asp Gly Leu Ala Leu Ala Leu



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	50		55		60											
	Glu	Pro	Ala	Leu	Ala	Ser	Pro	Ala	Gly	Ala	Ala	Asn	Phe	Leu	Ala	Met
	65					70					75					80
5	Val	Asp	Asn	Leu	Gln	Gly	Asp	Ser	Gly	Arg	Gly	Tyr	Tyr	Leu	Glu	Met
				85						90					95	
	Leu	Ile	Gly	Thr	Pro	Pro	Gln	Lys	Leu	Gln	Ile	Leu	Val	Asp	Thr	Gly
				100					105					110		
10	Ser	Ser	Asn	Phe	Ala	Val	Ala	Gly	Thr	Pro	His	Ser	Tyr	Ile	Asp	Thr
			115						120					125		
	Tyr	Phe	Asp	Thr	Glu	Arg	Ser	Ser	Thr	Tyr	Arg	Ser	Lys	Gly	Phe	Asp
15		130						135				140				
	Val	Thr	Val	Lys	Tyr	Thr	Gln	Gly	Ser	Trp	Thr	Gly	Phe	Val	Gly	Glu
	145						150				155				160	
	Asp	Leu	Val	Thr	Ile	Pro	Lys	Gly	Phe	Asn	Thr	Ser	Phe	Leu	Val	Asn
20				165						170					175	
	Ile	Ala	Thr	Ile	Phe	Glu	Ser	Glu	Asn	Phe	Phe	Leu	Pro	Gly	Ile	Lys
			180						185					190		
	Trp	Asn	Gly	Ile	Leu	Gly	Leu	Ala	Tyr	Ala	Thr	Leu	Ala	Lys	Pro	Ser
25			195					200					205			
	Ser	Ser	Leu	Glu	Thr	Phe	Phe	Asp	Ser	Leu	Val	Thr	Gln	Ala	Asn	Ile
		210						215					220			
	Pro	Asn	Val	Phe	Ser	Met	Gln	Met	Cys	Gly	Ala	Gly	Leu	Pro	Val	Ala
30		225				230				235					240	
	Gly	Ser	Gly	Thr	Asn	Gly	Gly	Ser	Leu	Val	Leu	Gly	Gly	Ile	Glu	Pro
				245					250					255		
	Ser	Leu	Tyr	Lys	Gly	Asp	Ile	Trp	Tyr	Thr	Pro	Ile	Lys	Glu	Glu	Trp
35			260						265					270		
	Tyr	Tyr	Gln	Ile	Glu	Ile	Leu	Lys	Leu	Glu	Ile	Gly	Gly	Gln	Ser	Leu
		275						280					285			
	Asn	Leu	Asp	Cys	Arg	Glu	Tyr	Asn	Ala	Asp	Lys	Ala	Ile	Val	Asp	Ser
40		290						295				300				
	Gly	Thr	Thr	Leu	Leu	Arg	Leu	Pro	Gln	Lys	Val	Phe	Asp	Ala	Val	Val
	305				310						315				320	
	Glu	Ala	Val	Ala	Arg	Ala	Ser	Leu	Ile	Pro	Glu	Phe	Ser	Asp	Gly	Phe
45				325						330					335	
	Trp	Thr	Gly	Ser	Gln	Leu	Ala	Cys	Trp	Thr	Asn	Ser	Glu	Thr	Pro	Trp
			340						345					350		
	Ser	Tyr	Phe	Pro	Lys	Ile	Ser	Ile	Tyr	Leu	Arg	Asp	Glu	Asn	Ser	Ser
50			355						360					365		
	Arg	Ser	Phe	Arg	Ile	Thr	Ile	Leu	Pro	Gln	Leu	Tyr	Ile	Gln	Pro	Met
		370						375				380				
	Met	Gly	Ala	Gly	Leu	Asn	Tyr	Glu	Cys	Tyr	Arg	Phe	Gly	Ile	Ser	Pro
55		385				390					395				400	

Ser Thr Asn Ala Leu Val Ile Gly Ala Thr Val Met Glu Gly Phe Tyr  
 405 410 415  
 Val Ile Phe Asp Arg Ala Gln Lys Arg Val Gly Phe Ala Ala Ser Pro  
 5 420 425 430  
 Cys Ala Glu Ile Ala Gly Ala Ala Val Ser Glu Ile Ser Gly Pro Phe  
 435 440 445  
 Ser Thr Glu Asp Val Ala Ser Asn Cys Val Pro Ala Gln Ser Leu Ser  
 10 450 455 460  
 Glu Pro Ile Leu Trp Ile Val Ser Tyr Ala Leu Met Ser Val Cys Gly  
 465 470 475 480  
 Ala Ile Leu Leu Val Leu Ile Val Leu Leu Leu Leu Pro Phe Arg Cys  
 15 485 490 495  
 Gln Arg Arg Pro Arg Asp Pro Glu Val Val Asn Asp Glu Ser Ser Leu  
 500 505 510  
 20 Val Arg His Arg Trp Lys  
 515

## 25 Claims

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the ASP1 polypeptide of SEQ ID NO: 2; or a nucleotide sequence complementary to said isolated polynucleotide.  
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2. The polynucleotide of claim 1 wherein said polynucleotide comprises the nucleotide sequence contained in SEQ ID NO: 1 encoding the ASP1 polypeptide of SEQ ID NO: 2.
3. The polynucleotide of claim 1 wherein said polynucleotide comprises a nucleotide sequence that is at least 80% identical to that of SEQ ID NO: 1 over its entire length.  
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4. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.
5. The polynucleotide of claim 1 which is DNA or RNA.  
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6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a ASP1 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.
7. A host cell comprising the expression system of claim 6.  
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8. A process for producing a ASP1 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.
9. A process for producing a cell which produces a ASP1 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a ASP1 polypeptide.  
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10. A ASP1 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO: 2 over its entire length.  
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11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO: 2.

12. An antibody immunospecific for the ASP1 polypeptide of claim 10.

13. A method for the treatment of a subject in need of enhanced activity or expression of ASP1 polypeptide of claim 10 comprising:

- (a) administering to the subject a therapeutically effective amount of an agonist to said polypeptide; and/or
- (b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the ASP1 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said polypeptide activity *in vivo*.

14. A method for the treatment of a subject having need to inhibit activity or expression of ASP1 polypeptide of claim 10 comprising:

- (a) administering to the subject a therapeutically effective amount of an antagonist to said polypeptide; and/or
- (b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said polypeptide; and/or
- (c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said polypeptide for its ligand, substrate, or receptor.

15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of ASP1 polypeptide of claim 10 in a subject comprising:

- (a) determining the presence or absence of a mutation in the nucleotide sequence encoding said ASP1 polypeptide in the genome of said subject; and/or
- (b) analyzing for the presence or amount of the ASP1 polypeptide expression in a sample derived from said subject.

16. A method for identifying compounds which inhibit (antagonize) or agonize the ASP1 polypeptide of claim 10 which comprises:

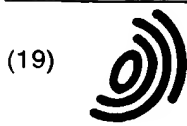
- (a) contacting a candidate compound with cells which express the ASP1 polypeptide (or cell membrane expressing ASP1 polypeptide) or respond to ASP1 polypeptide; and
- (b) observing the binding, or stimulation or inhibition of a functional response; or comparing the ability of the cells (or cell membrane) which were contacted with the candidate compounds with the same cells which were not contacted for ASP1 polypeptide activity.

17. An agonist identified by the method of claim 16.

18. An antagonist identified by the method of claim 16.

19. A recombinant host cell produced by a method of Claim 9 or a membrane thereof expressing a ASP1 polypeptide.





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(71) Applicants:  
• **SMITHKLINE BEECHAM CORPORATION**  
Philadelphia Pennsylvania 19103 (US)  
• **SMITHKLINE BEECHAM PLC**  
Brentford, Middlesex TW8 9EP (GB)

(72) Inventors:  
• **Powell, David J.**  
King of Prussia, Pennsylvania 19406 (US)

- **Southan, Christopher,**  
SmithKline Beecham Pharm.  
Harlow, Essex, CM19 5AW (GB)
- **Chapman, Conrad G.,**  
SmithKline Beecham Pharm.  
Harlow, Essex, CM19 5AW (GB)
- **Evans, Joanne R.,** SmithKline Beecham Pharm.  
Harlow, Essex, CM19 5AW (GB)

(74) Representative:  
**Crump, Julian Richard John et al**  
FJ Cleveland,  
40-43 Chancery Lane  
London WC2A 1JQ (GB)

(54) **Aspartic protease ASP1**

(57) ASP1 polypeptides aspartic proteases and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing ASP1 polypep-

tides and polynucleotides in the design of protocols for the treatment of Alzheimers's Disease, cancer, and melanoma, among others, and diagnostic assays for such conditions.

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# PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention EP 97 30 9648 shall be considered, for the purposes of subsequent proceedings, as the European search report

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
D,A	R. THOMAS TAGGART ET AL.: "Human pepsinogen C (progastricsin)" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 264, no. 1, 5 January 1989 (1989-01-05), pages 375-379, XP002136485 MD US * the whole document * -----	1-13,15, 16,19	C12N15/57 C12N9/64 C12N5/10 C12N1/21 C07K16/40 A61K48/00 A61K39/395 A61K31/70 A61K38/48 C12Q1/37
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
			C12N C07K A61K C12Q
INCOMPLETE SEARCH			
<p>The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.</p> <p>Claims searched completely :</p> <p>Claims searched incompletely :</p> <p>Claims not searched :</p> <p>Reason for the limitation of the search:</p> <p>see sheet C</p>			
Place of search		Date of completion of the search	Examiner
THE HAGUE		27 April 2000	MONTERO LOPEZ B.
CATEGORY OF CITED DOCUMENTS			
<p>X : particularly relevant if taken alone</p> <p>Y : particularly relevant if combined with another document of the same category</p> <p>A : technological background</p> <p>O : non-written disclosure</p> <p>P : intermediate document</p> <p>T : theory or principle underlying the invention</p> <p>E : earlier patent document, but published on, or after the filing date</p> <p>D : document cited in the application</p> <p>L : document cited for other reasons</p> <p>&amp; : member of the same patent family, corresponding document</p>			

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INCOMPLETE SEARCH  
SHEET C

Application Number  
EP 97 30 9648

Although claim 13 is directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compounds.

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Claim(s) searched completely:  
1-12, 15, 16, 19

Claim(s) searched incompletely:  
13

Claim(s) not searched:  
14, 17, 18

Reason for the limitation of the search:

Present claims 17 and 18, 13(a) and 14(a) relate to an extremely large number of possible compounds. Support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC is not to be found, however, for any specific example of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, no search has been carried out for those parts of the claims which are directed to agonists and antagonists, namely claims 17 and 18 and 13(a) and 14(a).

Present claim 14(b) and 14(c) relate to a molecule defined by reference to a desirable characteristic or property, namely inhibiting the expression of a nucleotide sequence and competing with a polypeptide for its ligand, substrate or receptor. The claim covers all molecules having this characteristic or property, whereas the application does not provide support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC for any specific example of such molecules. In the present case, the claim so lacks support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claim also lacks clarity (Article 84 EPC). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, no search has been carried out for claim 14, parts (b) and (c).

